

translation of the claims as filed with the International Application. This is a page for page substitution resulting in the deletion of the multiple dependency of claims. Please further amend the attached substitute claims as follows:

IN THE CLAIMS

Page 36, please delete the title at the top of the page which reads "Novel protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines".

Claim 16, line 1, change "claims 13, 14, or 15" to --claim 13--.

Claim 17, lines 6 and 7, change "one or more of the preceding claims" to --claim 1--.

Claim 19, lines 1 and 2, change "one or more of the preceding claims" to --claim 1--.

Claim 21, line 2, delete " 14 or 15,".

Claim 23, lines 7 and 8, please change "one or more of the preceding claims" to --claim 1--.

Claim 27, lines 4 and 5, please change "one or more of the preceding claims" to --claim 1--.

Respectfully submitted,

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Novel protein having at least differentiation-inducing
activity on Friend erythroleukemia cell lines

C L A I M S

1. Protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines having the following properties wherein said protein has to comply with at least the features a), b), c), and d):
 - a) may be isolated from murine myelomonocytic leukemia cell lines;
 - b) may be isolated from irradiated human bone marrow stromal cell lines;
 - c) induces differentiation in Friend erythroleukemia cell lines with hemoglobin formation;
 - d) having a molecular weight in the range of about 10 - 60 kDa as determined by gel filtration on Sephacryl S300®;
 - e) with an expression of the corresponding mRNA in primary cells of the thymus, fetal liver, adult spleen, or bone marrow;
 - f) with characteristic repeat structures in the cDNA encoding the protein;
 - g) with corresponding mRNA species of different length consisting of identical 3' regions but different 5' regions.
2. Protein according to claim 1, characterized in that said protein has at least one of the following features:
 - h) showing a stable in vitro expression of the corresponding mRNA if an allogenic spleen cell reaction is carried out with non-irradiated, not pretreated spleen cells of mouse strains CBA and C57Bl/6;
 - i) having AT rich regions in the cDNA encoding the protein;
 - k) inducible by a serum factor present in fetal calf serum.
3. Protein according to claim 1, characterized in that

one or more of the repeat sequences presented in Table 3 or of repeat sequences hybridizing to these repeat sequences under stringent conditions are present in the DNA encoding the protein of claim 1 or claim 2.

4. Protein according to claim 1, characterized in that said protein may be isolated from human cells, murine cells, or the culture supernatants of human or murine cell lines.
5. Protein according to claim 1, characterized in that said protein exhibits a partial amino acid sequence encoded by a DNA hybridizing to the cDNA of SEQ ID NO:1 or NO:2 or NO:4.
6. Protein according to claim 5, characterized in that said protein exhibits a partial amino acid sequence encoded by a DNA hybridizing to the cDNA of SEQ ID NO:1 or NO:2 or NO:4 under stringent conditions.
7. Protein according to claim 1, characterized in that there are also comprised portions, analogues, and derivatives of said protein as well as fusion proteins each coding for a protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines.
8. Protein according to claim 1, having an essentially purified, native form.
9. Protein according to claim 1 having an essentially recombinant form.
10. Protein according to claim 1 said protein having at

least differentiation-inducing activity on Friend erythroleukemia cell lines and/or growth factor activity and/or colony-stimulating activity.

11. Protein according to claim 1, characterized in that said protein exhibits a differentiation-inducing effect on human leukemia cell lines.
12. Protein according to claim 1, characterized in that said protein contains partial amino acid sequences according to SEQ ID NO:3 or NO:5 wherein one or more of the amino acids may be deleted, substituted, or added each having at least differentiation-inducing activity on Friend erythroleukemia cell lines.
13. DNA fragment according to SEQ ID NO:1 or NO:2 or NO:4 or the complementary strand thereof, portions, derivatives, and analogues thereof each coding for a polypeptide having at least differentiation-inducing activity on Friend erythroleukemia cell lines.
14. DNA fragments, portions, analogues, and derivatives thereof each coding for a polypeptide having at least differentiation-inducing activity on Friend erythroleukemia cell lines hybridizing to the cDNA according to SEQ ID NO:1 or NO:2 or NO:4 and/or which are degenerated by the genetic code.
15. DNA fragments, portions, analogues, and derivatives thereof each coding for a polypeptide having at least differentiation-inducing activity on Friend erythroleukemia cell lines hybridizing to the cDNA according to SEQ ID NO:1 or NO:2 or NO:4 under stringent conditions and/or which are degenerated by the genetic code.

16. DNA fragment of claims 13, 14 or 15,
characterized in that
said DNA fragment encodes at least a part of a
polypeptide with the activity of the human or murine
protein having at least differentiation-inducing
activity on Friend erythroleukemia cell lines according
to one or more of the preceding claims.
17. Recombinant vector,
characterized in that
said vector contains a DNA sequence corresponding to a
gene or a DNA fragment encoding the protein with at
least differentiation-inducing activity on Friend
erythroleukemia cell lines according to one or more of
the preceding claims.
18. Recombinant vector according to claim 17,
characterized in that
said vector is derived from a bacterial plasmid, a
bacteriophage, or a viral vector.
19. Host cell transformed by a vector according to one or
more of the preceding claims.
20. Host cell according to claim 19,
characterized in that
said host cell is a prokaryotic cell or an eukaryotic
cell.
21. Method for the preparation of a DNA fragment according
to claim 13, 14 or 15,
characterized in that
said fragment comprises screening of a human or murine
cDNA clone library using as a probe a DNA fragment of a
DNA coding for a murine or human protein having at least
differentiation-inducing activity on Friend
erythroleukemia cell lines.

22. Monoclonal or polyclonal antibody directed against at least one epitope of a protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines according to claim 1.
23. Therapeutic, diagnostic or experimentally useful means, characterized in that
said means contains as an effective substance at least one nucleic acid in an effective amount which hybridizes to a gene or a part thereof encoding the protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines according to one or more of the preceding claims.
24. Means according to claim 23,
characterized in that
said means contains as an effective substance at least one nucleic acid comprising (a) the nucleotide sequence encoding a protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines, (b) a portion thereof, (c) a nucleotide sequence hybridizing to a nucleic acid as under (a) and/or (b) under stringent conditions, or (d) a nucleotide sequence complementary to a nucleotide sequence as under (a), (b), and/or (c).
25. Means according to claim 23,
characterized in that
said nucleic acid optionally is a modified DNA.
26. Means according to claim 23,
characterized in that
said nucleic acid optionally is a modified RNA.
27. Therapeutic means,
characterized in that
said means contains a protein, an analogue, a derivative or portions thereof according to one or more of the

preceding claims each functioning as a polypeptide with at least differentiation-inducing activity on Friend erythroleukemia cell lines together with conventional carriers and excipients in an effective amount.

28. A molecular probe in diagnostics or therapy comprising a means according to claim 23.
29. An antisense nucleic acid for the inhibition of gene expression comprising a means according to claim 23.
30. DNA encoding a protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines, a portion, derivative, or analogue thereof each functioning as a polypeptide with at least differentiation-inducing activity on Friend erythroleukemia cell lines for the incorporation into a prokaryotic or eukaryotic cell.
31. Fusion protein having an amino acid sequence consisting completely or in part of the amino acid sequence of the human or murine protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines according to claim 1 and in part of a prokaryotic and/or eukaryotic protein.
32. Synthetic protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines according to claim 1 and having an amino acid sequence at least part of which is encoded by a DNA sequence hybridizing to the DNA sequence according to SEQ ID NO:1 or NO:2 or NO:4 at least under stringent conditions.
33. A protein according to claim 1 or inhibitors of said protein for the treatment of diseases in which a local or systemic overproduction or underproduction of this protein affects the development of the disease or the course thereof.

34. A protein according to claim 1 as a growth factor, colony-stimulating factor, a factor inducing erythropoiesis and/or inducing the immune system.
35. Protein according to claim 1, characterized in that said protein comprises at least those amino acids which are encoded by nucleotides 74 - 154 or 155 - 685.